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ON HEALTH SYSTEMS

II. THE DANISH SYSTEM

By JOHANNES FRANDSEN

In the future as in the past, mutual edification while discussing problems of common interest must be a prominent feature of international health co-operation — and its arrangement will therefore be one of the most important spheres of work of the World Health Organization.

Co-operation of this kind must necessarily presuppose that those taking part have a rough knowledge of how the various member countries manage these affairs.

With this in view, in a previous article,*) I referred to the desirability of getting as many countries as possible to publish reports on their public health legislation and the principles on which their organization and administration were based, as well as on their working results in order to provide a means of appraising the efficiency of the various health systems.

Reports of this kind should be drawn up on broad lines within a reasonable compass and give references to enable those more interested to provide themselves with more detailed information.

As an example of what I mean I shall try to describe the Danish health system — not solely for the sake of the example, however, but also because I believe in all modesty that there will be aspects and features of general value and interest in this centuries-old section of Danish social life, evolved side by side with the country's cultural life as a whole.

From the National Health Service.
Director-General: *Johannes Frandsen.*

*) Dan. Med. Bull. 1955, *2:* 193.

History.

The history of the Danish health system is a long one, but I shall make it brief.

With its roots striking down into the ideas of liberty and human rights and dignity born with the French Revolution, an epoch-making reform legislation saw the light in the years around 1800.

In Denmark this took place without a revolution, being accomplished in an absolute monarchy by the farsighted counsellors of the Crown. The democratic form of government was not introduced until 1849.

At the turn of the nineteenth century the reforms first and foremost embodied the emancipation of the lower classes, the peasants, who were transformed into free, independent farmers. Another reform was the introduction of compulsory education for children.

Characterized by the same spirit and at the same time, the foundations were laid for a public health system by means of health legislation which may be described as very comprehensive for the time.

The affairs and social status of apothecaries and physicians had already been established by law in 1672, and the year 1740 saw the appointment of a medical organ, advisory to the government and with certain administrative tasks. But we had no real health system until about 1800.

The first epidemic law was given in 1782, supplemented in 1805 with a quarantine law. In 1790 it was made compulsory for anyone suffering from venereal disease (syphilis) to seek treatment for it; in return, the treatment was free. After this came the law of compulsory vaccination against smallpox, given in 1810. Different in character, but no less important, was the hospital

law of 1806, which charged the local authorities, the counties, with the duty of building and maintaining a suitable number of hospitals. The social placing and administrative basis of the hospital system were thus laid down: the hospitals became public, communal.*)

The prolific development of bacteriology and surgery in the decades round about the year 1900 created new and heavy demands for access to hospital treatment. A system of health insurance, founded in 1892, provided the financial resources for utilizing to the full the results of medical science and applying them in the service of medicine for the population at large. Hospitals were enlarged and modernized and new ones were built. A campaign against tuberculosis was begun at the turn of the century and legalized in 1905. Sanatoria were established. The period was one of care for the sick — of treatment of disease.

Developments have been most fruitful and profound during the past quarter century. The hospital system has constantly expanded and adapted itself to the demands made upon it. Sickness insurance has been revised and brought up to date.

Nevertheless, a particularly important feature of this period was the founding of a general, preventive health legislation which, in contrast to the social legislation, embraces the entire population regardless of income and wealth. The outstanding section of that legislation is concerned with the health control of expectant mothers and of children up to their fifteenth year.

The duties and rights of physicians have been established by revising and renewing the laws. We are trying to adapt the work of the general practitioners outside the hospitals to the work in the hospitals — and vice versa — and the practitioner is being made the central figure in preventive health control.

In the same period, industrial hygiene was growing rapidly, resulting in a revision of the existing laws to be completed in the present year, including an extension of the original working basis into a comprehensive workers' protection with the collaboration of a special corps of physicians.

Concurrently with the development and growth of the health work as roughly outlined above, its organization and administration were expanded and, as regards its medical aspects, became more crystallized in form with the establishment of a collective, central organ of medicine.

I. ORGANIZATION

The Danish health system comprises both treatment and preventive as well as health-promoting measures. It is administered partly by the State and partly by local, communal authorities and is financed out of fiscal revenues by means of grants from Parliament and from communal councils.

a) *Treatment.*

The hospital system generally is maintained and run by the communes. Under the Acts of Parliament it works on grants from county and town councils, supplemented by subsidies from the State (at present about half) and is administered by these councils jointly.

The communal councils fix the hospital charges; comprising entire hospital attention with examinations and treatment, these charges must be sanctioned by the Ministry of the Interior. In no case do the present charges cover more than about a fourth of the actual working cost, often less, and the public health insurance (the sick clubs), which pays for its members, even have a reduction of 50 per cent.

Apart from the public hospitals there are only few private institutions; they are under public supervision and work intimately with the public hospital system.

Insanity is treated at special hospitals which, outside of Copenhagen, are maintained by the State. The City of Copenhagen runs its own mental hospital system.

The treatment of disease outside the hospitals is in the hands of general practitioners and specialists. This form of medical attention to members of the sickness insurance is paid for by the clubs at rates agreed upon by negotiation between the insurance and the medical organizations and approved by the Ministry of Social Affairs.

The right to practise medicine is open to all registered physicians. Special authority is required for assuming the title of specialist.

The public sickness insurance comprises about 85 per cent of the population and provides its members with medical attention and a part of the medicine while at home, hospital treatment, dental treatment, help in confinement, etc.

b. *Infectious diseases.*

Tuberculosis patients are treated in sanatoria, which with State subsidies are run by the National Association for the Fight against Tuberculosis, or in communal tuberculosis hospitals which simultaneously form the centres of a system of chest-clinics covering the entire country. These hospitals and clinics are worked by county and town councils with State subsidies as in the case of the general hospitals. BCG vaccination is offered free of charge to all tuberculin-negatives, the expense being defrayed by the State.

It is the duty of patients with infectious venereal diseases to submit themselves to treatment, which in return is given free by publicly appointed physicians. Patients attending a private

*) The word "communal" here is used in the sense that in Denmark the local government areas are known as "communes".

physician must be reported to the local health officer.

Epidemic diseases are treated free of charge at special hospital departments under the communal hospital system. The cost is refunded by the State. Local, communal epidemic commissions and quarantine commissions take the necessary steps to restrict and combat epidemics as they arise.

Vaccination.

Public vaccination against smallpox, which is practically compulsory, and free of charge, is performed by the local health officers at State expense.

Diphtheria vaccination is offered free to all under the age of 18. It is paid for by the State, administered by the communes and performed by general practitioners.

Vaccination against poliomyelitis is being completed at the quickest possible rate commensurable with the State Serum Institute's output of the vaccine. To the fullest extent this vaccination will be carried out according to similar principles as diphtheria vaccination and will be offered to all under the age of 40.

c) *Health control.*

The condition of healthy pregnant women can be checked up free of charge by a physician — the family doctor or any other medical man at their option — at 3 consultations and by the midwife at 6 consultations during their pregnancy and 1 after the birth.

For infants in their first post-natal year the parents can obtain supervision by specially trained public health nurses and have that supervision supplemented by professional examination (usually the family doctor) at 3 consultations. Health control of a similar kind by physicians is given to children after their first post-natal year until their school-entering age, with 1 consultation per annum.

The public health nurses are appointed by the communes with a fixed annual wage, of which half is refunded by the State. Doctors and midwives are paid by the State for every examination according to the fees fixed by the Ministry of the Interior after consultation with the Danish Medical Association and the Midwives Association.

All schools, public and private, must appoint school doctors so that all children are under health control during their school years up to the age of about fifteen. About half the schools in the country have school dentists; a shortage of qualified dentists prevents an obligatory school dental arrangement.

School doctors, who are appointed by the local school authorities, must be approved by the Ministry of Education. Specialist physicians applying for these posts are given preference, but the great majority of school doctors are general practitioners, and at intervals of a few years they are called in for short courses of instruction on subjects connected with children's diseases and hygiene.

d) *Industrial hygiene.*

Resulting from a radical revision in 1954 of the laws concerning inspection of factories and workplaces, there is now under the Ministry of Social Affairs an all-round state labour inspectorate which also comprises a special corps of physicians and an affiliated institute of labour hygiene.

e) *General hygiene.*

There is separate legislation concerning food control and housing hygiene, obliging State organs to take direct action. But in the widely ramified field of housing hygiene and the ever-growing sphere of food control the highest responsibility now, lies with the communes as it did when the first law on health byelaws was passed in 1858.

The communal councils, town and parish councils, frame the health byelaws which, after receiving the approval of the Ministry of the Interior, have the force of law within the single communal area. These byelaws include, inter alia, regulations as to housing, water supply, food control, schools, cemeteries.... Health byelaws give the Health Committees the executive authority to carry out their provisions.

Health byelaws have the same objects and in principle the same means, but in spheres of operation they extend from the metropolis with its 800,000 inhabitants to the little rural parish with a few hundred people.

f) *Pharmacies.*

The preparation and distribution of medicines now, as it has done for almost 300 years, proceed according to fixed rules. Medical remedies may only be distributed through the pharmacies. These pharmacies work under strict control, a control which also covers industrially manufactured preparations.

II. ADMINISTRATION

For important spheres of its work the public health system is decentralized. It is the communal authorities, the councils and their administrative organs, that bear the responsibility. This is true of the purely administrative part of the work, but it is particularly true of the budgeting for those sections of the health system, inter alia, the hospitals, which are dependent upon grants by the local councils.

Centrally too the fields of the health system are in several different hands. The Ministry of the Interior, which is the ministry for the communes, administers the greater part of the health

legislation that is entrusted to the communes, but the Ministry of Social Affairs watches over the public sickness insurance in addition to industrial hygiene, and the Ministry of Education administers the school doctor system. Other large and small administrative spheres of importance and value to the public health system come within the province of other ministries, for example: the health of the military forces is in the care of the Ministry of Defence.

Thus the questions might justifiably be asked: How can co-operation between the various bodies be upheld?

Where is the organization whose job it is to assemble and knit together the many threads and, based upon a profound insight into the many fields and the wide expanse of public health work, ensures unity within this plurality and the possibility of progress and growth in complete harmony — thereby creating a public health system? And where is the medical component that is the prime condition of the organization and existence of a public health service?

Not in a Ministry of Health, not in a separate health department within an existing ministry, but outside of the central administration proper and yet intimately associated with it, this organization and this medical component are present in an independent institution: The National Health Service (Sundhedsstyrelsen).

The placing and scope of this central institution, laid down in a special Act of Parliament, will best be visualized by quotations from that Act:

§ 1. The central administration of the country's public health system shall be called The National Health Service.

§ 2, 1. The National Health Service shall be the chief supervisor of public health and nursing, including dental surgery, midwifery and pharmacies, and chief adviser to the public in all matters requiring a knowledge of medicine or drugs. In this capacity the National Health Service shall be consulted by the various departments of the central administration on all matters whose decision is considered by the ministry concerned to require such knowledge — including budgetary questions of this kind. The National Health Service shall likewise on request by the said authorities give opinions on matters of such nature.

§ 2, 2. The scope of the National Health Service shall, as regards health and nursing, also comprise matters relating to the Army and Navy .... as well as Greenland.

§ 3, 1. The National Health Service shall closely follow the state of health in the country and see that public health legislation is observed, draw the attention of local authorities to infringements and defects and receive communications as to matters of public health which may be directed to it by authorities or organisations, and, when deemed necessary, submit suitable recommendations for improvement. Furthermore, the National Health Service shall, whenever occasion demands, take steps to circulate guidance — in print or verbally — with regard to special health measures or special circumstances relating to disease.

§ 3, 2. The National Health Service shall have .... chief supervision of .... all State and local curative institutions and nursing institutions and private institutions of similar kind .... The opinion of the National Health Service shall be required as to plans for the building of or for extensive alterations to the said institutions. The permission of the Minister of the Interior for the building or extension of private hospitals shall be required.....

§ 4, 1. All physicians, dental surgeons, midwives, nurses, masseurs and the like and all druggists and their assistants are subject to the authority of the National Health Service.....

§ 6. The National Health Service shall arrange and direct the collection and analysing of the medical statistical material, the results of which shall be published by it.....

The head of the National Health Service must be a physician.

Otherwise, the personnel of the National Health Service comprises physicians, nurses, pharmacists and administrative people (jurists). Besides this staff, occupied with the usual health objects of hygienic and disease-combating character, there is a separate department of nutritional hygiene, and of particular importance is the department of medical statistics with a statistician at its head. The Service also has attached to it a number of counsellors, representing the various branches of medical science and selected from among university professors and leading physicians-in-chief at the hospitals. Besides these scientific counsellors this staff of advisory experts includes a hospital administrator, a professor from the Technical University (technical hygiene), a judge of the Supreme Court and representatives of the professional organizations of physicians, dental surgeons, nurses and midwives.

Other institutions connected with the National Health Service are the State Serum Institute, the State Vitamin Laboratory and an institute for pharmaceutical research. A separate institute for nutritional research is in the course of preparation.

Under it the National Health Service has a staff of medical officers of health (county and district), 67 in all. Their position and scope are determined by an Act of Parliament. They represent the National Health Service vis-à-vis the local councils, supervise matters of hygiene with the right and duty to reprimand and are ex officio members of the local health committees.

There are separate laws for physicians and dental surgeons, midwives, nurses and pharmacists, laying down rights and obligations. This whole medical personnel ranges under the National Health Service "in so far as concerns their office, occupation, transactions and obligations as such" and they must provide "such notifications and reports as may be required of them in the interests of public health". When appoint-

ments are to be made to leading positions at the hospitals, the qualifications of the applicants must be adjudicated upon by the National Health Service before the post is filled.

It is not by direct, independant administration of public health institutions that the National Health Service exercises its most important function. Its purely administrative activities appear more in the background.

Its all-important influence on the working and development of the public health system lies in its central position in an advisory capacity to both the Central Administration and the communal councils. The duty of the departments of State to call for a National Health Service opinion on all matters affecting health, the duty of the local councils to co-operate similarly with the health officers and the latters' reports to the Service on such matters, as well as returns from physicians, hospitals, etc., enable the National Health Service at all times to exercise complete surveillance over the various administrative branches of public health, central as well as local. This provides the service with the solid basis for its co-ordinating activities and for utilizing its right and duty to map out the lines for harmonious development and to promote this by sumbitting proposals for the emendation and expansion of the existing legislation and administrative orders and for the necessary grants.

III. STATUS

Then what have we achieved, and how far have we got in building up the comprehensive treatment machinery which the public health system must have in the fight against infectious diseases and in the purely health-preserving work?

This is not the place for making a long and detailed report; but a mainly numerical synopsis will, I hope, enable an impression to be formed of the efficiency of our health system.

Denmarks has an area of 43,018 sq.km with 4,369,000 inhabitants.

In 1953 the country had 4,750 physicians, distributed over the following spheres:

General practioners	1911
Practising specialists	550
Physicians at hospitals and other curative institutions	1981
School doctors (mainly G. P.'s)	449
Local health officers (the majority with the right to practice)	67
Dental surgeons	1900
including school dentists	200
" hospital dentists	6
Midwives	759
Nurses	about 15000

	1928	1953
Birth rate per 1000 pop.	19.6	17.9
Total mortality per 1000 pop.	11.0	9.0
Mean expection of life at birth:		
males years	60.9	67.8
females "	62.6	70.1

Curative institutions.

Medical-surgical hospitals are established all over the country, with at least one large Central Hospital with various special departments in every county, besides smaller hospitals (50—150 beds), mostly with a surgeon at the head, some with both medical and surgical departments and an X-ray department. Apart from 2 State hospitals (one a university clinic in Copenhagen) and a few private ones, all the hospitals are communal.

Medical-surgical hospitals:

	Number	Number of beds in all	Number of beds per 1000 pop.
1928	154	16,527	4.7
1953	146	24,552	5.6

This section of the hospital system is in a state of constant development as to both premises and organization. The country fully understands the duty of the health system to keep abreast of the times, to keep pace with the progress of medical science and medicine so that they may be utilized to the full and offered to all, regardless of place of residence and wealth. The annual expenditure on building is high and hitherto the expense budgets have been on the increase. At the present time the running costs of all medical-surgical hospitals total Kr. 300 mill. (about Kr. 40.— per bed-day and about Kr. 70.— per head of the population).

The tuberculosis institutions in 1929 had beds corresponding to 1.0 per 1000 of the population, against 0.9 in 1953. As a result of the decrease in tuberculosis this figure will be further reduced in the near future. A large number of beds are now empty, so that some of these curative institutions will have to be closed down and arranged for the treatment of other categories of patients.

The mental hospitals had in

1928 a total of 6,553 beds, or 1.9 per 1000 pop.
1953 " " " 9,497 " , " 2.2 " " "

There is a need for more accommodation and a building programme for the future is under consideration, combined with plans for some rearrangement and modernization.

The curative institutions proper had in 1928 a total of 26,170 beds, representing 7.6 per 1000 pop. In 1953 the corresponding figures were 38,105 and 8.7.

These figures are exclusive of the Asylums for feeble-minded, which at present have 7,103 beds or 1.6 per 1000 of the population, plus a similar number of places under family care.

Infectious diseases.

Tuberculosis

	1928			
	new notified cases		deaths	
	total	pr. 100,000	total	pr. 100,000
Pulm. TB.	3,878	111	1904	54
Other TB.	—	—	696	20
All TB.	—	—	2600	74

	1953			
Pulm. TB.	1,764	40	339	7.8*)
Other TB.	219	5	45	1.0
All TB.	1,983	45	384	8.8*)
*) in 1954: ..			310	7.0
			31	0.7
			341	7.7

It must be added that in the same period bovine tuberculosis was vigorously dealt with. The fight was waged in conjunction with the dairy farmers' organization and financing and with the skilful aid of the veterinary surgeons. Since 1953 there have been no tuberculin-positive cattle in Denmark.

Venereal diseases.

	1928 notified cases		1953 notified cases	
	total	per 100,000	total	per 100,000
Gonorrhoea	11,989	343	7,551	173
Acq. syphilis ..	1,927	55	149	3

Epidemic diseases.

	1928 notified cases		deaths	
	total	per 100,000	total	per 100,000
Typhoid fever	133	4	22	0.6
Diphtheria	5,752	164	190	5.0
Scarlet fever	2,378	68	21	0.6

	1953 notified cases		deaths	
	total	per 100,000	total	per 100,000
Typhoid fever	31	0.7	1	0.02
Diphtheria	9	0.2	1	0.02
Scarlet fever	3471	79.0	0	0

HEALTH CONTROL

Increasing numbers have taken advantage of the offer to all pregnant women of free medical examination at 3 consultations, supplemented by 7 consultations with midwives. Last year 91 per cent of all expectant mothers came to the doctors for the first consultation, but only 72 per cent completed all 3 and only 58 per cent used the opportunity to see the midwives.

As yet the supervision of first-year children by public health nurses comprises only 60 per cent of the newly-born. Chiefly owing to a shortage of fully trained public health nurses only a corresponding part of the communes have so far opened appointments for these nurses. In both town and country these nurses are well thought of and are welcomed in the homes as good friends and highly appreciated advisers. Last year 98.6 per cent of the homes with newly born children accepted the nurses' offer of visits and help.

	1928	1953
Infant mortality per 1000 live-born	81	27

The offer of free medical consultations for healthy children until they reach school-attending age has not yet been utilized to the same degree. Last year the percentage was only 53.

The cost of this health control and of school doctors in 1954 was about Kr. 17 mill.*)

*) £ 1 = kr. 20. Direct comparison on this basis is impossible, of course. The purchasing power of the currency at home does not always correspond to its exchange value.

POLIO VACCINATION IN DENMARK IN APRIL—JUNE 1955

I. THE PRODUCTION OF FORMALINIZED POLIOVACCINE AND PRELIMINARY RESULTS

By HERDIS von MAGNUS, PREBEN von MAGNUS, INGER PETERSEN, ANNELISE GODTFREDSEN and MOGENS RØNKJÆR

The epidemics of poliomyelitis have presented serious problems in Denmark for many years, but the epidemic in the fall of 1952 with a total of 2450 paralytic cases was the most severe in the history of the country (Fig. 1). (1, 2).

The work with a formalinized polio vaccine which was carried out by Salk and co-workers was consequently followed with particular interest here. As soon as the promising results with vaccination in humans had been published (3) is was decided to start a production of vaccine, closely following the methods described.

The results of the large field trial in the U.S.A. were published in April, 1955 (4). This study, comprising the vaccination of about 420,000 children against poliomyelitis, presented solid evidence that a formalinized vaccine prepared as described by Salk and co-workers (5, 9) was innocuous and reasonably effective.

In Denmark it was therefore decided to start polio vaccination immediately, following the plans worked out beforehand. Accordingly, vaccination against polio with a vaccine manufactured in this laboratory was made available for all children in the first five grades in school (ap-

From Statens Seruminstitut, Copenhagen.
Director: *J. Ørskov.*

This study has been aided by grants from Landsforeningen mod Børnelammelse (Polio) and from Hans McKinney Møllers Mindefond til Bekæmpelse af Børnelammelse.

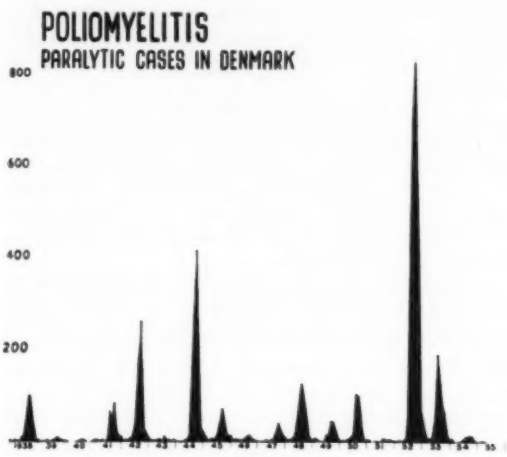

Fig. 1.
Monthly incidence of paralytic poliomyelitis in Denmark, 1938—1955.
Number of paralytic cases per month

proximately 7—12 years of age). The vaccination program started on April 25, 1955. The organization of this program, which resulted in the vaccination of approximately 425,000 children, is described in an accompanying report (10).

It is the purpose of the present paper to give a short description of the production of the vaccine used this spring, including the tests performed for safety and antigenicity at that time. The technique used for inoculation of the vaccine will be described. An account will be given of preliminary antibody studies in a small group of children. The present epidemic status in the country will also be mentioned.

MATERIAL AND METHODS*)

Cultivation of virus for vaccine.

Tissue cultures prepared from trypsinized monkey kidney tissue were used for growing polio virus. The trypsinization technique employed is described in an accompanying paper (11).

A suspension of trypsinized cells was introduced into Roux flasks together with 150 ml of growth medium. This medium consisted of "Medium 199" (12) containing 1 per cent horse serum plus antibodies, 100 U. penicillin and 100 U. streptomycin per ml (see accompanying paper). After 6—7 days of incubation at 37°C., the glass after gentle rinsing of the flask with 50 ml "Medium 199", a fresh growth medium consisting of "Medium 199" with antibiotics as above but without horse serum was introduced.

*) From the very beginning of our work with a formalinized polio vaccine, we received generous help and advice from Dr. Salk as well as from his co-workers, Dr. Youngner, Dr. Bazeley, Major Bennett, and Dr. Lewis. Without their assistance the present Danish polio vaccination program would not have been possible.

The flasks were now seeded with 0.3 ml of tissue culture virus and re-incubated for 3—5 days at 37°C. At this time the cells were found to be completely destroyed. Each flask was examined individually in the micrescope. The fluids were now harvested and stored at +4°C. (or ÷ 12°C.) until the processing of the vaccine took place.

Virus strains.

The virus strains selected to represent polio virus Type 1, 2 and 3, respectively, were the Brunhilde, MEF-1 and Saukett strains.

The MEF-1 and Saukett strains were received from Dr. Salk's laboratory and are identical with the strains used by the manufacturers of polio vaccine in U.S.A. The tissue culture passage of the Brunhilde strain originated from Dr. Ender's laboratory and had been obtained in 1953 in its 10th tissue culture passage from Dr. Arne Svedmyr, Stockholm.

Measuring of virus activity.

Tubes with outgrown trypsinized monkey kidney cells were used for virus titrations. The culture medium at the time of virus inoculation consisted of 1.8 ml of straight "Medium 199" containing 100 U. of penicillin and 100 U. of streptomycin per ml. Groups of 5 tubes were inoculated with 0.2 ml of virus suspension diluted serially in steps of 0.5 log. The tubes were placed in a roller drum at 37°C. and examined microscopically after 4 and 7 days. Tubes showing definite signs of typical virus degeneration were considered positive for virus. From the 7-day readings the titers (ID_{50}) were calculated according to Kärber (13). They were based on the dilution of the virus suspension added to the cultures. No correction has been made for the small volume 0.2 ml used for inoculation.

Neutralization tests in tissue culture.

Serial 2-fold dilutions of serum were made in saline and mixed with equal volumes of virus. The virus dose was calculated so that the virus-serum mixture contained approximately 100 ID_{50} per 0.2 ml. After incubation for one hour at room temperature, 0.2 ml of the mixtures were inoculated into duplicate sets of roller tubes containing 1.8 ml of medium. The tubes were read microscopically after 7 days and the titer calculated on the basis of the 7-day reading. The various grades of degeneration were recorded as 1+ to 4+ as suggested by Ledinko and Melnick (14). The criterion for presence of neutralizing antibody was the capacity of a serum dilution to neutralize virus in 50 per cent of the tubes. A difference of at least 2+ between the virus control tubes (containing 100 TC doses but no serum) and a serum-virus tube was regarded as neutralization. The titer is given as the reciprocal of the highest serum dilution with this capacity.

Inactivation of virus.

Single strain pools were made in the amount of 19—20 liters in Pyrex bottles. In order to remove cell debris, this material was filtered through Corning tubular filters of fritted glass (No. 35000), using the 3 porosities: Coarse (40—60 microns), Medium (10—15 microns) and Fine (4—5.5 microns). A positive pressure of approximately 1 lb. per square inch was applied.

The filtration time varied from 2 hours to 9 hours and was in most cases about 5 hours. After filtration the bottle was placed in a waterbath at 37°C. and the excess of CO_2 in the medium was removed by aeration with filtered sterile air for an appropriate period of time, usually overnight. This procedure brought up the pH from 7.0 to around 8.4. The pH was re-adjusted to 6.9—7.0 by adding acetic acid, 0.5 n. To this preheated virus suspension was then added formalin to a final concentration of 1:4,000 of a 35 per cent solution of formaldehyde. (A dilution of 1:200 of formalin was used and added to the virus suspension in a proportion of 1:20). A continuous mixing during the addition of formalin was carried out by bubbling sterile air through the virus suspension.

The mixture was then transferred to a fresh bottle and placed in the incubator at 37°C. for 9 days. The bottle was rocked gently for mixing once a day during the inactivation period. On the 5th day, the contents were transferred to a fresh bottle in order to secure thorough mixing of the fluid and equal distribution of formalin throughout the bottle. At the end of the incubation period, the container was placed in a cold room at + 4°C.

An additional amount of antibiotics (100 U. penicillin plus 100 U. streptomycin per ml) was added to the vaccine either before or after inactivation, in a few instances in both cases.

Tests for virus activity during inactivation. Safety tests in tissue culture.

The original titers of the 20 liter virus averaged for type 1 $10 \div 6.50$, for type 2 $10 \div 6.60$ and for type 3 $10 \div 6.70$. After filtration the titers averaged $10 \div 6.40$, $10 \div 6.60$ and $10 \div 6.50$ for type 1, 2 and 3, respectively.

The tests for virus activity during inactivation were made in the following way: The first sample was taken for virus titration when the addition of formalin to the virus suspension had taken place and the mixture had been transferred to a new bottle. At this point the titer had dropped 0.3—0.6 log (aeration, heat inactivation and addition of formalin).

Another 150 ml sample was taken simultaneously and distributed in amounts of approximately 25 ml in vials which were sealed immediately. These vials were then incubated together with the large bottle, and twice a day for 3 days, at approximately 9 a. m. and 4 p. m., one vial was removed from the incubator. A 10 ml amount of fluid was dialyzed to remove the formalin, and a virus titration in roller tubes was carried out on the dialyzate.

In order to make sure that the virus activity in the large 20-liter bottle was identical with the activity measured in the vials during the first 3 days of inactivation, comparative titrations were occasionally carried out. The titers were found to be identical in the small and large containers in all instances.

After 3 days no virus could be demonstrated in the fluid by routine inoculation of roller tubes. A chart of the inactivation curves in seen in Fig. 2.

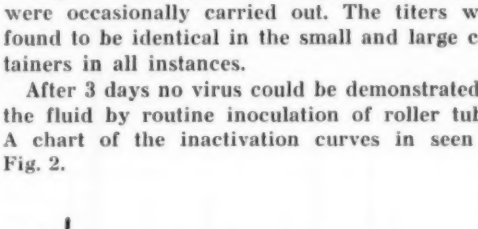

Fig. 2.
Rate of inactivation of polio virus.

The titer recorded at zero time is the virus activity measured after aeration and addition of formalin (see text for details).

The difference in inactivation rate for the 3 types of polio virus which is apparent in Fig. 2 is not systematic. In our present vaccine production the inactivation curves for all three types of polio virus resemble closely the type 3 curve shown here.

In the Minimum Requirements, Poliomyelitis Vaccine, Amendment No. 2, Dec. 14, 1954 issued by U. S. Public Health Service*) it was required to test each monovalent bulk strain pool for infectious virus before making the final polyvalent vaccine by inoculation of a sample consisting of 1 ml per liter of the pool into tissue culture bottles (further specifications are given in the Requirements). The bottles should be observed for at least 14 days and subcultures should be made after 7 and 14 days. Each lot of final trivalent vaccine was required to be tested in the same way.

*) This document as well as later issues have generously been made available to our laboratory. The regulations herein listed were followed with only few deviations during the manufacturing of polio vaccine in Denmark early this year.

Since in our country one laboratory is alone responsible for production as well as for the control of the vaccine, it was felt desirable to elaborate the above-mentioned tests somewhat This was done by performing two additional, identical tests which were carried out on samples withdrawn from the 20-liter bottle on the 5th and 7th day of inactivation. Also, two subcultures instead of one were made after 7 and 14 days. Furthermore, in all instances samples of 5 ml instead of 1 ml per liter fluid were tested.

By applying these tests it was demonstrated that although the 5-day sample occasionally contained traces of virus, the 7- and 9-day tests were consistently negative. It was felt that the 7-day negative tests added a certain margin to the safety tests. (As another supplement was added tests for active virus on each 5-liter container with final trivalent vaccine. This was done in order to be able to exclude accidental contamination of the final product with live virus).

Other tests for safety and innocuity.

The various additional tests described in the U.S.A. Minimal Requirements were carried out on a pilot sample of the final vaccine. This sample was made by mixing 100 ml of each of the inactivated monovalent pools assigned for the final trivalent pool and neutralizing the formalin in this mixture with sodium bisulfite.

This sample was used for intracerebral inoculation of 10—12 Rhesus monkeys and for intramuscular inoculation into 6 Cynomolgus monkeys in order to test for active polio virus. Ten mice were inoculated intracerebrally to test for lymphocytic choriomeningitis virus, and in addition two litters of newborn mice were inoculated peritoneally. The tests for mycobacterium tuberculosis as well as the test for B-virus in rabbits were done on this pilot sample.

The content of total nitrogen was measured and found to vary from 0.180 mg/ml to 0.196 mg/ml.

Tests for potency.

These tests were carried out in monkeys and in guinea pigs (15). Ten to sixteen monkeys weighing 3.0 kg to 5.5 kg were inoculated subcutaneously with 1 ml of vaccine 3 times one week apart. Blood was drawn one week after the last inoculation.

Guinea pigs weighing 250 g were inoculated intradermally with 2 injections of 0.05 ml each. Groups of 10 animals were inoculated with undiluted vaccine. This was done 3 times one week apart and the animals were bled one week after the last injection.

The serum neutralization tests were performed on the serum from each animal individually. The serum titers are listed as the geometric mean of these titers (Table 1).

Table 1.
Antibody response in monkeys and guinea pigs vaccinated with 3 pools of polio vaccine used in April—June 1955.

	Antibody Type	Serum antibody titer*) Monkeys	Guinea pigs A	Guinea pigs B**)
Pool 4	I	9	4	
	II	18	4	not done
	III	33	8	
Pool 5	I	10	4	
	II	7	4	not done
	III	12	3	
Pool 6	I	24	6	85
	II	55	11	105
	III	13	3	25
Reference Vaccine A (Pittsburgh)	I	—	3	52
	II	—	5	100
	III	—	4	32

*) Expressed as the geometric mean of the reciprocal of the highest serum-dilutions showing 50 per cent neutralization.

**) Tests listed under B were carried out on vaccine which had been stored at +4° C for 6 months (see text for details).

Unfortunately, the Rhesus monkeys used for immunization had recently been imported from India and were not in good health. Several of the monkeys died from pneumonia during the experiments. The antibody responses in the remaining animals were extremely varying.

The antibody response in guinea pigs was found to be lower than seen in previous studies and also in later experiments with other vaccines. That this finding was due to seasonal variations in the guinea pigs (probably vitamin deficiencies) and not to a particular low potency of the vaccine was indicated by the fact that Reference A vaccine, kindly supplied to us by Dr. S a l k, also at that time elicitated a low antibody response in our guinea pigs (Table 1).

After the completion of the various tests for antigenicity and safety, the final trivalent virus pools were made and the formalin content was neutralized by the addition of sodium bisulfite.

The vaccine used in the 1954 field trial in U. S. A. contained merthiolate as a preservative. On recommendation by Dr. S a l k we did not add any preservative to the Danish vaccine. As a control measure our vaccine batches were tested repeatedly for sterility during preparation. The pooled trivalent vaccine was filtered through glass filters (Coarse-Medium-Fine) and the distribution into ampoules took place as soon as possible after this filtration.

The vials were kept at room temperature for 2 weeks and checked individually for visible contamination before labelling and storage in the icebox. In spite of the lack of preservative, contaminated ampoules were found extremely rarely.

ADMINISTRATION OF THE VACCINE

The vaccine inoculations were given intradermally in the forearm with two injections of 0.1—0.15 ml each, this resulting in two papules with a diameter of about 8 mm. This procedure was repeated after 4—6 weeks, and a third injection will be given 9—12 months after the first injection.

Complications.

No cases of paralysis occurred in the vaccinated children. In fact, no case of paralytic polio occurred in Denmark during the spring and early summer. The reactions following the administration of the vaccine by the dermal route were mild and infrequent, consisting mainly in local swelling of the arm on the site of inoculation. A preliminary estimate indicates that this occurred in 0.1—0.2 per cent of the children. These data will be described and analysed in a forthcoming paper. In all instances where cases of illness (fever, headache and miscellaneous other symptoms) were suspected of being associated with vaccine inoculation, stool- and serum samples were obtained from the child. None of these stool samples from a total of 42 children have been found to contain polio virus when examined in tissue culture.

Antibody response in vaccinated children.

In order to study the antibody response in children and also to be able to follow the persistence of vaccination antibodies in the future, it was decided to collect sera from children from two sections of Copenhagen as well as from cities and rural districts in other parts of the country.

Blood samples were obtained before vaccination from approximately 2,300 children in the second grade of school. A preliminary screening of 2,100 of these pre-vaccination serum samples, using undiluted serum in neutralization tests in roller tubes, has been carried out. It was found that 13 per cent of these 8-year-old children had no antibodies to any of the 3 types of polio virus. Antibodies to all 3 types were found in 24 per cent of the sera.

Since the second inoculation of the vaccine was given 4 weeks later, right before the beginning of the school summer vacation, a post-vaccination sample was usually not taken until August or September. However, from a small group of children a post-vaccination sample was obtained 2 weeks after the second inoculation (6 weeks after the first one).

Since we were pressed for time, it was decided to examine first those samples which would be of greatest importance. Paired serum samples from 48 children who did not have any antibodies against Type 1 polio virus, were accordingly chosen for this preliminary study. In Fig. 3 the results of this investigation are shown in the same manner as employed in the report from the 1954 Field Trial in the U. S. A. (4).

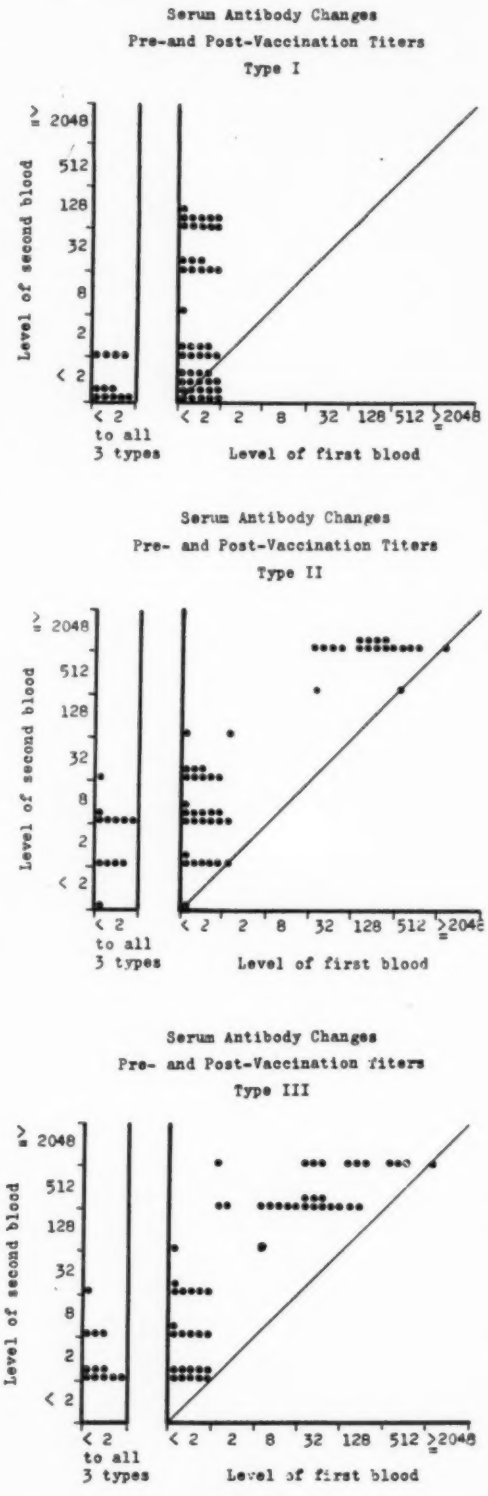

Fig. 3.
Serum antibody changes in a group of 48 children without type I antibodies before vaccination.

It will be seen that the response as regards formation of antibodies to Types 2 and 3 was good: Of 27 children without antibodies to Type 2 before vaccination all but one developed Type 2 antibodies, i. e., vaccination response to Type 2 occurred in 96 per cent of the children. As regards Type 3, all 23 children without Type 3 antibodies before vaccination showed Type 3 antibodies after vaccination. The response was thus 100 per cent. The Type 1 response was less satisfactory. Out of 48 children, who did not have Type 1 antibodies before vaccination, 29 children or 60 per cent developed antibodies to this type. Of 12 without antibodies to any of the 3 types, only 4 developed Type 1 antibodies after vaccination.

Salk has found that the pattern of the prevaccination antibody influences the response to primary vaccination (16). Although our data are very few, the same variation in antibody response can be demonstrated. In Fig. 4 the different prevaccination antibody patterns are compared with the vaccination response as regards formation of antibodies to Type 1. It will be seen that 20 out of 22 children with antibodies to Type 2 before vaccination developed antibodies to Type 1. Of those with antibodies to Type 3 only, or with no antibodies to any of the 3 types, 9 out of 26 showed antibodies to Type 1 after two doses of vaccine.

EPIDEMIOLOGY OF POLIOMYELITIS IN DENMARK in 1955

In Denmark the polio epidemics have regularly reached their peak in the first week of September, and the epidemic season now being over it may be said that this year has had an extremely low incidence of poliomyelitis. While the paralytic cases in 1952, 1953 and 1954 numbered 2450, 684 and 72, respectively, this year only 11 paralytic cases of polio have been reported from April through September.

On revision, the clinical diagnosis of paralytic polio was maintained for only 7 of these cases. In tissue culture tests, stool samples from 4 of these 7 patients were found to contain polio virus (two Type 1, one Type 2 and one Type 3), while the remaining 3 patients were not found to excrete polio virus. So far no cases of verified paralytic polio have occurred in vaccinated children.

Stool samples from a total of 125 patients who have been admitted to various Danish hospitals from April 1 through September under the diagnosis of lymphocytic meningitis or aparalytic polio have been examined in tissue culture and newborn mice. No strains of polio virus have been isolated from these patients. However, an appreciable number of Coxsackie- and Orphan viruses has been isolated from these patients (unpublished data).

DISCUSSION

The preparation of polio vaccine in this laboratory was based on the work with a formalinized vaccine carried out in Pittsburgh by Dr. Jonas Salk and his associates. The procedure described by these workers has been closely followed. The vaccines prepared in the U. S. A. and here through 1954 should accordingly be comparable with the following two exceptions: The vaccine prepared in this laboratory did not contain any preservative and the Brunhilde strain was selected to represent polio virus Type 1.

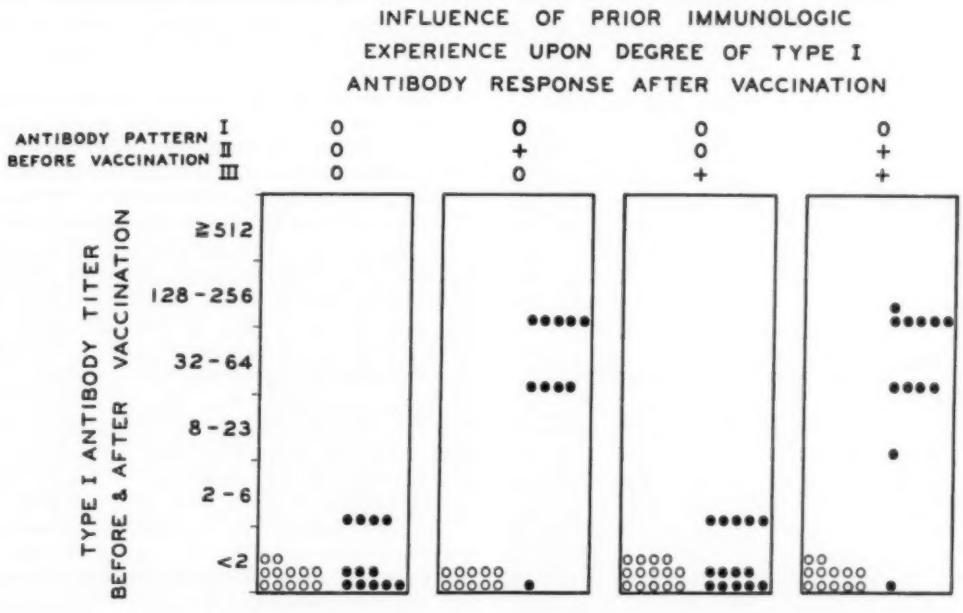

Fig. 4. *Titers before vaccination* ○. *Titers after vaccination* ●

The addition of preservative was omitted because of the observed destructive action on the virus antigen by merthiolate (8). The Brunhilde strain was preferred to the Mahoney strain because this latter strain was known to be particularly virulent for monkeys by peripheral routes (3, 17). In antigenicity studies in guinea pigs (15) the immunizing effect af the Brunhilde strain was found not to differ essentially from that of the Mahoney strain. The geometric mean of the antibody titers obtained in guinea pigs in 2 experiments by intradermal inoculation of undiluted virus were 1:104 for the Brunhilde strain and 1:182 for the Mahoney strain. The titers of the virus strains used for immunization were $10 \div 6.7$ and $10 \div 6.6$, respectively.

As regards the formalin inactivation of the vaccine, the rate of inactivation observed early in the year in this laboratory varied considerably from one batch to another (Fig. 2). A scrutinizing of the factors involved in the inactivation revealed that the inactivation rate was closely correlated with variations in the temperature of the incubators. A variation of 1 degree centigrade greatly influenced the inactivation curve. This observation has been elaborated in experiments with variations of the formalin concentration and of the temperature of inactivation (unpublished data).

The tests for safety which were performed in tissue culture and in monkeys on the Danish vaccine this spring and summer were the most sensitive available at that time. More sensitive tests for the presence of live virus are now being developed in various laboratories and modifications in filtration technics are being investigated. It may be expected that progress will be made continuously and that the procedure for large scale processing of a formalinized vaccine will be perfected.

As regards the use of guinea pigs for potency testing of polio vaccine, these animals have been found to be well suited by Swedish workers (15). These observations have been confirmed also in this laboratory. During the summer and fall of 1954 we thus obtained satisfactory titers and reproducible results using these animals in potency tests. However, during the winter months, the antibody response of the guinea pigs used for potency tests was very low. Also, calculation of the extinction limit values (18) met with difficulties at that time because of the irregularity in the response within the groups of inoculated animals. That the unsatisfactory results obtained during the winter season were not due to an inferior antigenicity of the vaccines then prepared was demonstrated by comparison with Reference A vaccine and by re-testing one of the batches 6 months later simultaneously with Reference A vaccine. At this time the antibody response in guinea pigs inoculated with undiluted vaccine was found satisfactory (Table 1, Section B). If results obtained with potency tests in guinea pigs should be comparable throught importance the year, it is accordingly of extreme to maintain the guinea pig colony in uniform condition. It seem probable that nutrition plays an important role in this connexion and that special care must be taken to avoid vitamin deficiences during the winter months (19, 20).

The background for the Danish vaccination program, which aimed at the vaccination of five age groups of children in the spring of 1955, was the successful polio vaccination trial in the U.S.A. in 1954 which was sponsored by the *National Foundation for Infantile Paralysis*. Only few changes from the American pattern were made here. However, the first series of vaccinations consisted of only 2 injections, 4 weeks apart and the inoculations were made intradermally and not intramuscularly.

The intradermal route of inoculation was chosen because it was considered to be the most appropriate route under the given circumstances. In Denmark most vaccines and other injections are by tradition given subcutaneously, and the intramuscular route of inoculation is used only when absolutely necessary. Intramuscular inoculations have been shown under certain circumstances to be capable of provoking paralytic poliomyelitis in humans (21, 22) and in monkeys (23). These facts together left the intramuscular route out of consideration.

The intradermal route was preferred to the subcutaneous route for the following reasons:

1. The intradermal route is generally recognized as being the route of choice for sensitization (24).
2. In animal experiments inoculations under the skin have been found capable of provoking paralytic polio, while trauma of the skin did not have this effect (23).
3. The use of a small inoculum seemed desirable for theoretical as well as for practical reasons, and it was to be expected that an inoculum of 0.2—0.3 ml given in the skin would elicit about the same immunity as an inoculum of 1.0 ml given intramuscularly (5).

As regards the antibody response in humans, this has so far been studied only in 48 children which were bled 2 weeks after the second inoculation of vaccine (Fig. 3). Whether this is the optimal time for observing an antibody response after intradermal inoculation is not known. Since the post-vaccination blood specimens were collected at varying times after the vaccination, some information on this point may perhaps be obtained when all the paired sera collected from the vaccinated children have been titrated.

The third injection of vaccine, the booster injection, will be given in the spring of 1956. At that time pre- and post inoculation blood samples will again be collected.

Our preliminary study on the vaccination anti-

body response in children indicates that while the Type 2 and Type 3 strains seem to have a satisfactory antigenicity under the given circumstances, it would be of advantage if a more potent Type 1 strain could be incorporated in the vaccine. In Pittsburgh studies on the antigenitiy and virulence of a number of Type 1 strains have been carried out for some time (16). Also other laboratories have been screening Type 1 strains in order to be able to incorporate a Type 1 strain with high potency and low virulence in the vaccine.

Altogether 425,000 children 7—12 years of age were vaccinated against polio in Denmark this spring. This number represents about 98 per cent of the children in these age groups. In a "normal" epidemic year the age distribution of paralytic polio cases should thus have indicated whether the 2 vaccine injections given had had any protective effect. However, from April 1 through September only 7 clinical cases of paralytic polio occurred in Denmark (only 4 of these patients were found to excrete polio virus). This small number, of course, does not allow any evaluation of the effectiveness of the two inoculations of polio vaccine.

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POLIO VACCINATION IN DENMARK APRIL—JUNE 1955

II. ORGANIZATION AND PROCEDURE FOR THE SCHOOL VACCINATION

By SVEND TULINIUS and E. JUEL HENNINGSEN

The epidemic of poliomyelitis which struck Denmark in the years 1952—1953 is classified as the largest since the first occurrence of epidemic polio in Denmark about fifty years ago, with 2450 cases of paralytic polio in 1952 and 684 cases in 1953. The epidemic starting in June, 1952 and running through April, 1953 comprised 2560 paralytic cases. More than half of the cases occurred in the metropolitan area, Copenhagen and suburbs, with one million inhabitants or one quarter of the total population in Denmark. During the epidemic running through the summer and autumn of 1953, only sporadic cases occurred in the Copenhagen area, while some of the counties not affected in the 1952 epidemic had a large number of cases in 1953. In 1952 70 per cent of the cases occurred among children below the age of 15. The age group most afflicted was that from 1—3 years. The epidemics were also characterized by the severity of clinical illness, about 20 per cent of the paralytic cases were bulbar, and the fatality rate of bulbar cases was 50 per cent.

From The National Health Service,
Director-General: *Johs. Frandsen* and
Statens Seruminstitut,
Director: *J. Ørskov.*

GENERAL CONSIDERATIONS

This dark background had to be kept in mind when in the winter of 1954 a vaccination programme was made for the best possible use of a limited amount of vaccine prepared at Statens Seruminstitut according to the method described by Salk and co-workers. The vaccination programme had to be planned in all details prior to the decision as to whether the vaccination should be carried out or not. The final decision could not be made till after the report on the 1954 poliomyelitis vaccine field trial in the United States had been published.

The amount of vaccine available was limited; it was rather late in the spring to fulfil the first two injections before the polio season started. There was general agreement that a real field trial was unthinkable, but, on the other hand, it was considered most important to get as much information as possible concerning the effectiveness of the vaccine. It was therefore decided to offer vaccination to the school children in the first 5 classes, that is 7—12 years of age. By doing this it was possible to establish a "controlled" vaccination of children already organized and regularly supervised by school doctors. It was possible to get a solid registration of the vaccinations and to collect a reasonable number of blood samples before and after vaccination in order to get an impression of the antibody response.

By choosing these age groups and giving the vaccination at the schools, it was easier to avoid difficulties in the distribution of the limited amount of vaccine, anticipating a hard pressure on the physician from parents who wanted their children protected.

After careful consideration, it was decided to administer the vaccine by giving two intradermal injections of 0.1—0.15 ml each and repeating the procedure after 4—6 weeks. A third vaccination will be given after 9—12 months. Intramuscular injections of vaccines seem under certain circumstances to provoke paralytic polio, and as it had never been in use in our country for inoculations, it was out of the question. In animal experiments subcutaneous injections have been found able to provoke paralytic polio. Intradermal inoculations are known to give a high degree of sensitization with even small doses of antigen. For several reasons it was therefore desirable to give the vaccine intradermally. By using a double injection of 0.1—0.15 ml each, the risk of all the vaccine being given subcutaneously was greatly reduced.

ORGANIZATION OF THE VACCINATION

The school vaccination was organized through the medical officers of health headed by the epidemiological department of Statens Seruminstitut in collaboration with the National Health Service and the Ministry of the Interior.

Full instructions for the vaccination campaign were handed to the health officers at a meeting at the end of March, 1955, where they had an opportunity to discuss various questions. According to the number of children to be vaccinated in their district, the health officers had to appoint, in cooperation with the local branch of the Danish Medical Society, a number of school doctors and general practitioners to perform the vaccination at the schools. All necessary equipment for the vaccination, syringes, needles, boilers, forceps etc., was supplied through Statens Seruminstitut.

Through the Ministry of Education a circular had to be sent to all schools. A letter signed by the director general of the National Health Service, Dr. Johs. Frandsen, and the director of Statens Seruminstitut, Dr. J. Ørskov, had to be distributed through the schools to all parents of children in the first five classes. This letter contained information about the polio vaccination, and a parental request form to be signed and returned to the schools was attached to the letter.

Registration.

A scientifically made trial of the vaccine with reliable control groups with or without placebo injections was infeasible, for one thing because the control group would simply disappear within a short time because of the progression of the vaccination. The only reliable information it will be possible to get is the knowledge of cases of paralytic polio occurring in this vaccinated group in the years to come. Experience shows that it is extremely difficult to get absolutely reliable information whether a person has gone through a full vaccination or not. It therefore was necessary to make a registration. This could be made by setting up a special vaccination registration file of the vaccinated children, more than 400,000. This method is very costly and with the relatively high immunity level, developed as a result of the 1952—53 epidemics which these age groups experienced, only a relatively small number of cases — a few hundred — can be expected, even if the vaccination is without any effect. The vaccination registration, therefore, was made on their school registration cards with a special stamp. These school registration cards are official and are kept for a very long period. In the years to come there will be no difficulty in checking every notified case of paralytic polio among children of the age groups vaccinated in this special school vaccination. The vaccination certificate issued to each child may be of help in deciding whether full vaccination has been given, but in many cases they will have disappeared.

The whole plan with instructions for health officers and assistant doctors, the distribution of instruments and other material, circular letters, the message to parents with request forms, appointment of doctors and assistants, question of payment to doctors and assistants, and all other financial questions had to be solved and ready prior to taking the final decision. The whole vaccination plan necessarily had to be kept secret to a certain degree, in order to avoid too much public disappointment if the evaluation of the U. S. 1954 field trial should not justify a large scale vaccination, comprising 10 % of the whole population of Denmark.

THE PLAN IN OPERATION

On April 12th it was evident that the result of the U. S. field trial was positive, and the next

day the Ministry of Interior secured the sanction of the Treasury for the commencement of anti-poliomyelitis vaccination of children attending the first five classes of all schools in Denmark.

Now the plan could be put into operation, and on the 25th of April the first vaccinations in the schools were performed. Vaccination clinics were set up in more than 4000 schools and 600 doctors were giving the injections. In about 3 weeks the first round was finished and a week later the next started. The vaccination had to be finished before the summer holidays, which in most schools began on June 20th.

In planning the vaccination, it was calculated that of the 433,000 children in the first five classes hardly more than 400,000 would participate. The campaign, however, was met by the public, parents and children, with such interest that about 98 % of the children in question were vaccinated. This was an extremely high percentage, especially remembering that the normal absenteeism from school in springtime is about 10 %.

Blood sampling.

In order to get information about the antibody response to the vaccination and to follow the persistence of antibodies, blood samples were collected from 12 different rural or provincial town districts and from two different districts of the city of Copenhagen. About 2200 blood samples were taken before vaccination. From a smaller group of children — about 360 — a second sample was taken before the second inoculation and from approximately 225 a sample was taken 2 weeks after the second inoculation. From the other 2000 children the blood samples to be taken after second inoculation, because of the summer holidays beginning shortly after that injection, had to be postponed till August-September when the school reopened after the holiday. This might give a too optimistic and less reliable picture of the antibody development following vaccination, especially if there happened to be widespread infection. Fortunately this does not seem to be the case. So far this year there have only been very few sporadic cases of polio.

Material and sterilisation.

A few words must be said about the material used. For the injection, 1 ml Record glass metal syringes with interchangeable barrels were used. A special hypodermic needle made of non-corrosive steel no. 18, length 16 mm was used. This needle is 5 mm longer than those generally used for intradermal injections. The greater length makes injection easier. The syringes and needles were generally sterilized by boiling in distilled water, for the first time 15 minutes and later on at least 10 minutes. Some of the doctors had facilities for dry heat sterilization and preferred this. To avoid or minimize the risk of transferring infections, especially serum hepatitis, each syringe had to be sterilized after injecting 6 children, and the hypodermic needles were flamed between each child and not removed from the syringe before flaming, in order to prevent backflow into the syringe. Animal experiments had shown that the risk of transferring infections by needles and syringes was considerably reduced by using the intradermal technique and not removing the needles between the injections but sterilizing by flaming. There is no evidence that infection of any kind has been transferred during the whole vaccination campaign.

FUTURE POLIO VACCINATION POLICY

This first polio vaccination campaign was organized and headed by the Health Service, and the third inoculation of this group of school children is planned to be given in a similar school campaign 11 to 12 months after the first inoculation. But all other future polio vaccinations of other groups of children and adults will be done according to the principles laid down in a special Anti-Polio Vaccination Act of May 24, 1955: "The vaccination is voluntary and free of charge for all persons under 40 years of age. The vaccination will be performed by all general practitioners, who will receive their fee from the State. In accordance with the amount of vaccine available, the Minister of the Interior will decide which age groups gradually will become entitled to the vaccination".

Up till now the vaccination has been given free for all children from 9 months to 12 years of age.

In Greenland this summer, the total population was given the opportunity of vaccination.

In the Faroe islands all children at school had the opportunity of being vaccinated this summer.

Till now no case of paralytic poliomyelitis has occurred among persons vaccinated.

TISSUE CULTURES OF TRYPSINIZED KIDNEY CELLS FROM DIFFERENT MONKEY SPECIES

OBSERVATIONS ON THEIR SUITABILITY FOR POLIO VIRUS WORK

By PREBEN von MAGNUS, K. BIRKUM PETERSEN, VIGGO BECH, INGER PETERSEN and HERDIS von MAGNUS

The manufacturing of a polio vaccine in this institute required the establishment of a production of suitable tissue cultures. It was decided to employ trypsinized dispersed cells from monkey kidneys (2) and the technique followed was essentially the one described by Youngner (11).

Several successful preparations were obtained, employing this method, but in a number of cases the cell yield was irregular and on the whole rather low. Various modifications in technique which were tried in order to increase the yield will be discussed in this paper. The trypsinization technique finally adopted was a combination of the method described by Youngner and the revised procedure of Rappaport and Melnick (6, 9).

In the beginning only kidneys from Cynomolgus monkeys were used. But as the supply of these animals was scarce, experiments were carried out to determine whether kidney tissue from Rhesus monkeys would be equally efficient for the production of polio virus in high concentration. This was found to be the case (4). Consequently, tissue from Rhesus monkeys was also employed. Similar observations have been made by Farrel et al. (3).

Later on when difficulties were also encountered in obtaining Rhesus monkeys in sufficient numbers, various species of African monkeys were used.

The results obtained by the trypsinization of kidneys from 6 different species of monkeys will be discussed in this paper.

Studies on the sensitivity to polio virus of cells from the various species and on the amount of virus produced in the cultures will also be reported.

PREPARATION OF TRYPSIN DISPERSED CELL SUSPENSIONS

As has been mentioned, the technique employed is essentially a combination of the procedures described by Youngner (11) and by Rappaport (6, 9). The kidneys are removed aseptically from monkeys exsanguinated under sodium pentobarbital anesthesia. The capsule of the kidneys and as much as possible of the pelvis and the calyces are removed. The remaining tissue is cut with scissors into pieces about 2—3 mm in diameter. The minced tissue is weighed, then transferred into an Erlenmeyer flask and washed several times with PBS*). The tissue clumps are then poured into a 500 ml Erlenmeyer flask, indented in four places at right angles to each other and perpendicular to the bottom surface of the flask (6, 9). A magnetic bar covered with glass and protected with rubber tubing is placed in the flask and 200 ml of preheated (35°C.) trypsin**) is added. The "extraction" flask is placed in a waterbath, and a magnetic stirrer is placed under the waterbath with the flask. The motor is adjusted to a speed which gives maximum mixing without foaming. Mixing is continued for 5 minutes. The flask is then removed from the waterbath and placed for 1—2 minutes on the table to allow the tissue clumps to sediment. The supernatant containing the liberated cells is filtered through 2 layers of loosely woven gauze into a 2 liter Erlenmeyer flask which is kept in ice water to arrest the action of the trypsin.

Extraction is then continued by adding 200 ml of fresh trypsin to the remaining tissue clumps, replacing the extraction flask in the waterbath and by replacing the extraction flask in the waterbath and turning on the magnetic stirrer. The number of extractions required to exhaust the kidney tissue varies between 16 and 26 — depending on the amount of tissue in the preparation.

The pooled trypsinization fluids are centrifuged at low speed, i. e. 400 r. p. m. (24 x gravity) for thirty minutes. The supernatant fluid is discarded and the packed cells are resuspended in lactalbumin enzymatic hydrolyzate (7) prewarmed to 37°C. containing 2 per cent horse serum, penicillin (100 u. per ml) and streptomycin (0.1 mg pr. ml). The cells are resuspended in a total of 50 ml for each kidney used. Only if the kidneys are very small (average weight less than 2 g) or very big (more than 8 g) the amount of rhesuspend-

From Statens Seruminstitut, Copenhagen.
Director: *J. Ørskov.*

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*) Phosphate buffered Saline (11).

**) 0.25 % in PBS (Difco (1:250) or Nutritional Biochemicals Corporation (1:300)).

ing medium is decreased or increased, respectively. Rhesuspension is carried out by means of a Pasteur pipette, the cells being drawn up and expelled several times to loosen up aggregates. The stock suspension is made up in an Erlenmeyer flask with a magnetic bar and is stirred for 5—10 minutes to ensure that the suspension is homogeneous. A small sample is then withdrawn and the contents of nuclei determined in a Bürger-Türcks hemocytometer (6, 9). To 0.5 ml of the cell suspension is added 1 ml of 0.1 per cent crystal violet in 0.1 ml citric acid. Both cells which are apparently intact, i. e., nuclei surrounded with cytoplasm, and free nuclei are counted. However, when calculating the proper dilution of the stock for the preparation of tubes or the amount of stock to be added to the culture flasks, only the cell count of apparently intact cells is considered. If, for instance, the cell count of the stained suspension (which is a 1:3 dilution of the stock (see above)) is 100 cells per 0.1 mm^3, then 1 ml of the stock contains:

$$100 \times 10^4 \times 3 = 3 \times 10^6 \text{ cells.}$$

Culture tubes: Tubes 10 cm long, diameter 13 mm are used. Each tube is seeded with 1.2 ml lactalbumin hydrolyzate with 2 per cent horse serum containing 200,000 cells.*)

Roux-flasks: 1000 ml Roux flasks with a plane surface of 13 cm × 24 cm are seeded with 10—11 million cells. An amount of 150 ml of "synthetic mixture 199" (8) prepared according to Salk, Youngner and Ward (10) with 1 per cent horse serum is added to each flask*).

Incubation: takes place at 37°C. The outgrowth in the tubes is usually confluent in the tubes after 5—6 days, in the flasks after 6—7 days of incubation. During the preliminary incubation the tubes are placed in a slightly slanting position in closed boxes.

Comparative studies on the trypsinization procedures described by Youngner and Rappaport.

Using a revised trypsinization procedure, Rappaport (6, 9) reported cell yields 2 to 3 fold higher than obtained by Youngner. It was consequently thought of interest to compare the two methods in this laboratory. On two separate occasions one preparation of both kinds was made simultaneously. The results obtained with these 4 preparations are recorded in Table 1 which shows the average number of nuclei (total, surrounded by cytoplasm and "free" nuclei) per kidney and per gram of kidney tissue. As can be seen, there was no significant difference in the cell yields of the two preparations made on the same day. The very low cell yields obtained in Experiment 1 were probably due to the use of an inferior batch of trypsin (see below). Limitations in laboratory space and the necessity of continuous large scale production prohibited further experiments of this kind. It was, however, found advantageous for practical reasons to adopt a number of the modifications suggested by Rappaport. Thus, the specially designed "extraction" flask in combination with a magnetic stirrer has been found convenient. With such a flask it is easier both to control the stirring and to decant the fluid. The simplified centrifugation procedure of Rappaport saves considerable time, but as will be discussed later, the percentage of intact cells was found to be of the same order as in suspensions which had been subjected to repeated centrifugations at 1000 or 1500 r. p. m. The standardization of the stock suspension by cell count has been found very convenient and reliable, whereas the use of optical density measurements as used by Youngner (11) in our hands led to rather confusing results.

Miscellaneous variations in technique.

As has been mentioned above, irregular and low cell yields were encountered during earlier experiments. The low yields seemed to be correlated with the presence in the cell suspension of slimy substances, probably enclosing a large proportion of the liberated cells. A series of modifications in technique have been tried to avoid the occurrence of this "agglutination" phenomenon. Thus the period of time for each extraction with trypsin was varied from 3 to 10 minutes. This did not influence the slime-formation, but periods of 5 minutes were found optimal for giving maximal cell liberation with the smallest quantity of trypsin. The use of a waterbath (32°—34° C.) during

*) The culture fluids also contain 100 u. penicillin and 0.1 mg streptomycin per ml.

Table I.
Comparison of cell yields obtained by trypsinization of Rhesus kidneys by the methods described by Youngner and Rappaport.

Exp. no.	Trypsinization-method	Kidneys			Yield of nuclei					Intact cells	
		total number	Weight		total x 10^6	with cytoplasm		without cytoplasm		per kidney x 10^6	per gram kidney x 10^6
			total g	per kidney g		x 10^6	per cent	x 10^6	per cent		
1	Youngner	6	30	5.0	8.73	5.10	44	3.63	56	0.85	0.17
	Rappaport	4	23	5.8	5.15	3.80	43	1.35	57	0.95	0.17
2	Youngner	2	7	3.5	6.04	3.07	51	2.97	49	1.54	0.44
	Rappaport	2	7	3.5	5.88	3.29	56	2.59	44	1.65	0.47

Table II.
Comparison of cell yield and growth of cells from trypsinized preparations of kidneys from 6 different monkey species.

Monkey species	Number of preparations	Kidneys		Nuclei yield						Tissue-culture tubes (200,000 cell per tube)			Tissue culture flask (10 mill. cells per flask)		
				per kidney			per g kidney								
		total number	average weight g	total x 10^6	with cytoplasm x 10^6	without cytoplasm x 10^6	total x 10^6	with cytoplasm x 10^6	without cytoplasm x 10^6	prepared no.	confluent cell-layer 6' day no.	confluent cell-layer 6' day per cent	prepared no	confluent cell-layer 7' day no.	confluent cell-layer 7' day per cent
Guenon (cercopithecus)	10	239	3.5	1.57	0.96	0.62	0.45	0.27	0.18	6636	6581	99.2	447	435	98.5
Rhesus	10	354	4.4	2.39	1.45	0.93	0.54	0.33	0.21	7649	7514	99.2	845	834	99
Cynomolgus	4	114	5.2	2.55	1.55	1.00	0.49	0.30	0.19	3046	3046	100	258	256	99
Mangabey (cercecebus)	10	66	6.2	3.18	2.00	1.19	0.51	0.32	0.19	3825	3825	100	1143	1110	97.5
Mantled Baboon (Papio Hamadrias)	6	26	7.9	4.26	2.65	1.61	0.53	0.35	0.18	975	975	100	286	279	97.5
Dog-faced Baboon (Papio cynocephalus)	10	36	9.1	4.70	2.79	1.91	0.52	0.31	0.21	6994	6932	99.1	835	831	99.5

extraction possibly somewhat reduced the aggregation in the stock suspension and was found convenient because of the reproducibility of the working conditions. The brand of trypsin employed was found to be of considerable importance. Good results with almost no clumping were consistently obtained with trypsin preparations from Difco[1]) or Nutritional Biochemicals Corporation[2]), whereas products from a Danish firm apparently varied in quality from batch to batch. In some cases the cell yields were found equal to those obtained with the American products mentioned above, but frequently slime formation was abundant and the cell yield very low. Storage of the trypsin solution (0.25 per cent) at +4°C. for 1 week resulted in a loss of about 10 to 15 per cent of trypsin activity, but this did not significantly interfere with the trypsinization process.

Variation in centrifugation speed (from 200 to 1500 r. p. m.) was not found to be significant in influencing the tendency to cell aggregation nor the yield of "intact" cells.

[1]) Difco Laboratories, Inc., Detroit 1, Michigan.
[2]) Nutritional Biochemicals Corporation, 21010 Miles Avenue, Cleveland 28, Ohio.

Resuspending of the sedimented cells in a slightly acid medium (pH 6.6—6.8) increased the formation of slime. The phenomenon was less pronounced when resuspension was carried out in a medium with a pH of 7.2 to 7.4. More alkaline reactions had no further effect. Agglutination was more pronounced when the cells were resuspended in a cold medium than in a medium prewarmed to 37° C. However, among the various factors studied, the quality of the trypsin would seem to be the most important for the agglutination phenomenon.

The use of lactalbuminhydrolyzate (7) with 2 per cent horse serum for culture media in tissue culture tubes has been found to give definitely better results than the use of synthetic mixture 199. Still better results were obtained when the tubes were kept in closed boxes during the first 5 days of incubation intead of in open racks.

Cell yields obtained from different monkey species.

The cell yield obtained from kidneys of 6 different monkey species by the trypsinization procedure described is listed in Table II. The

Table III.
Sensitivity to polio virus Type I, II and III of kidney tissue from different monkey species. Titrations in roller tubes prepared from trypsinized kidney cells.

Monkey species	Type I				Type II				Type III			
	Virus: V-1548		Virus: V-2064		Virus: V-1549		Virus: V-2184		Virus: V-2065		Virus: V-2380	
	Exp. no.	Titer — log	Exp. no.	Titer*) — log	Exp. no.	Titer — log	Exp. no.	Titer*) — log	Exp. no.	Titer*) — log	Exp. no.	Titer — log
Guenon	—	—	2	6.80	—	—	1	6.65	1	≥6.45	—	—
Rhesus	1	6.55	7	6.48	1	7.35	7	6.30	14	6.53	1	6.95
Cynomolgus	—	—	—	—	—	—	—	—	2	6.63	—	—
Mangabey	1	6.45	—	—	1	7.25	—	—	3	6.45	1	6.95
Mantled Baboons	—	—	—	—	—	—	—	—	1	6.65	—	—
Dog-faced Baboons	—	—	3	6.38	—	—	3	6.48	10	6.79	—	—
Max. difference in titers	—	0.10	—	0.42	—	0.10	—	0.17	—	0.34	—	0.00

*) Geometric mean titers.

proportion of apparently healthy cells to "free" nuclei was about the same in all cases (60 per cent and 40 per cent, respectively). The number of cells obtained from one kidney varied considerably with the animal species. The yield obtained from dog-faced Baboons was thus 3 fold higher than that obtained from Guenons. However, looking at the yield as calculated per gramme of kidney tissue, it will be seen that there is hardly any difference in the yields from the six monkey species studied.

The right hand part of Table I lists the number of tubes and flasks prepared from the various preparations and the number and percentage of tubes and flasks showing confluent growth after incubation for 6 and 7 days, respectively.

Again, there is but little variation in the results obtained with cells from the various species studied. The discarding of a small percentage of flasks was mostly due to infection with moulds or other micro-organisms.

Observations on the sensitivity to small amounts of polio virus and on the yield of virus obtained from cells of the different monkey species.

The sensitivity of the kidney cells from the six monkey species was studied. Repeated titrations of 2 stock suspensions of each of the three types of polio virus, from time to time, have been carried out in roller tubes. The titration technique employed is described in an accompanying paper (5). The results are recorded in Table III.

Only one stock suspension of virus (V-2065, Type 3) has been titered in tissue from all six monkey species. The variation in the geometric mean titer was 0.34 log. The greatest variation in geometric mean titer in these series was 0.42 log observed for V-2064, Type 1.

The yield of virus in flask cultures has been recorded in Table IV. In all instances Roux flasks with a confluent layer of cells and containing 150 ml of "Parker 199" were seeded with 0.3 ml of undiluted tissue culture virus. Incubation was carried out at 36°—37°C. for 3—5 days. The fluids were harvested at the time of maximum cell degeneration, each flask being examined microscopically. Titrations have been carried out on pools from a varying number of Roux flasks.

Table IV.
Concentration of virus in single strain pools from flask cultures prepared from trypsinized kidney cells of different monkey species.

Monkey species	Polio virus types I		II		III	
	Exper. no.	Titer*) — log	Exper. no.	Titer — log	Exper. no.	— log Titer
Guenon	1	7.1	1	7.1	1	6.6
Rhesus	10	6.6	6	6.8	6	6.8
Cynomolgus	26	6.5	—	—	—	—
Mangabey	2	6.3	2	6.5	1	6.4
Dog-faced Baboon	2	5.9	6	6.1	5	6.6

*) Geometric mean titers.

From the data listed in Fig. 4 it may be seen that in our laboratory Rhesus, Cynomolgus and possibly Guenons have given the highest virus yields. In contrast, the virus concentration obtained by using kidney tissue from dog-faced Baboons has been varying and on the whole rather low.

DISCUSSION

The procedure for trypsinization of monkey kidneys employed in this laboratory has been based on the method described by Youngner (11), but some modifications suggested by Rappaport and Melnick (6, 9) have been adopted. While a number of smaller variations in technique did not appreciably influence the cell yield, the brand of trypsin used for "extraction" was found to be of importance. Trypsin from a Danish firm thus gave irregular and on the whole rather low cell yields. When trypsin preparations from two different American firms were used, the results were regular and fairly satisfactory.

In a few experiments Youngner's trypsinization method was compared directly with the revised procedure described by Rappaport. The cell yields obtained by either method were comparable. The reason for our failure in obtaining the high cell yields reported by Rappaport and Melnick (6, 9) remains obscure. The total number of nuclei (i. e., free nuclei plus nuclei surrounded by cytoplasm) in our Rhesus preparations amounted to 2.4×10^8. This figure is 2.0 to 2.5 times lower than the yield of intact cells obtained by Rappaport. The discrepancies can thus hardly be due to destruction of the cells, for example caused by the higher centrifugation speed employed in our standard procedure (400 r. p. m. = $24 \times g$), but would seem to be due to some loss of cells during the preparation.

As already mentioned, a distinctly better cell outgrowth was observed when the tissue culture tubes were placed in closed boxes instead of in open racks. The explanation may probably be that the frequent opening of the incubator used for the tubes resulted in temperature variations which were harmful to the cell growth in the tubes placed in open racks.

The yield of cells per gramme kidney tissue and the cell growth of trypsinized kidney cells from six different monkey species were found to be identical. The preference of one kind of monkey to another for tissue culture work may accordingly be a question of availability of the species, economical factors and the susceptibility of the cell cultures to the virus to be studied. As regards economisal factors, it should be pointed out that for a laboratory receiving the animals "unconditioned", right after they have been imported, the actual price of the animals is not the only circumstance to be considered. The general health of the animals plays an important role. During a 2 month period after the arrival of the

monkeys in this laboratory, the following approximate mortality rates from infectious diseases have been observed in spite of extensive treatment with antibiotics*): Rhesus: 40 per cent, Cynomolgus: 30 per cent, Guenon: 20 per cent, Mangabey: 15 per cent, Baboons (mantled and dog-faced): 2 per cent.

Studies on the cytopathogenic effect of polio viruses on fibroblast cultures prepared of testicles from various kinds of African monkeys have been previously reported by Barski and collaborators (1). These authors obtained excellent results with cultures prepared from various species of Guenons, Mangabeys and other African monkeys. The findings of these authors agree well with those reported in this paper. Comparative titrations of polio virus in cell cultures from the various monkey species employed in this study indicated that the different cultures were equally sensitive to limiting infectious doses of all three types of polio virus.

The virus yield obtained in flask cultures of cells from Baboons, however, was irregular and often lower than the yield obtained in cell cultures from Guenons, Rhesus, Cynomolgus and Mangabeys. The single cells of the kidney from Baboons are considerably larger than are the cells from the other kinds of monkeys mentioned. Accordingly, a confluent layer of Baboon kidney cells contains fewer cells than does a similar layer of cells from the other species. It seems probable that this difference in the actual number of cells present in the flasks may be responsible for the lower yield of virus obtained in cultures prepared from kidneys of Baboons.**)

SUMMARY

1. This paper describes the trypsinization procedure for the preparation of monkey kidney cell suspensions as employed in this laboratory. The effect of various modifications in technique is described. A comparison of the Youngner trypsinization method with the revised procedure described by Rappaport and Melnick is also reported.

2. The cell yield and cell growth of kidney cells from six different monkey species have been compared. The cell yield per gramme of kidney tissue was found to be identical. The percentage of tissue culture flasks and tubes showing confluent cell growth after incubation for 7 and 5 days, respectively, was not significantly different.

3. Studies on the sensitivity to limiting doses of polio viruses Type I, II and III indicated a comparable degree of sensitivity of the kidney cells from all six species of monkeys employed. The amount of virus produced in flask cultures was of the same order in cell cultures derived from Rhesus, Cynomolgus and Guenons. The virus yield was, however, unsatisfactory in cultures of kidney cells from Baboons.

*) Penicillin, streptomycin, Aureomycin and Chloromycetin.

**) *Addendum:* In Institut Pasteur, Paris, a satisfactory virus concentration has been obtained in kidney cells from Baboons using equal parts of "synthetic mixture 199" and Hank's solution as tissue culture medium. (Personal communication from Professor Lépine).

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VIII INTERNATIONAL CONGRESS OF PAEDIATRICS
Copenhagen 1956

The VIII International Congress of Paediatrics will be held in Copenhagen, Denmark, July 22—27, 1956.

The scientific program will comprise plenary sessions, round-table conferences, sectional meetings, and an exhibition.

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Monday, July 23rd: I. Prenatal Injuries and Malformations. II. Chemical Agents.
Tuesday, July 24th: I. Pathology of the Newborn: Anoxia. II. Surgery.
Wednesday, July 25th: I. Child Psychiatry. II. Neurology.
Thursday, July 26th: I. Infections. II. Allergy.
Friday, July 27th: I. Problems in Tropical Paediatrics. II. Tuberculosis.

Proffered papers will be read in sectional meetings. It is planned to arrange round-table conferences on eight different subjects.

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POLIOMYELITIS IN GREENLAND

By MOGENS FOG-POULSEN

TOPOGRAPHY AND POPULATION OF GREENLAND

Greenland is situated northeast of the North American continent and extends between the north latitudes 59° 46' (Cape Farvel) and 83° 39' (Cape Morris Jesup) and between the west longitudes 11° 39' (the Northeast Foreland) and 72° 08' (Cape Alexander). The area of the country is 2700 km from north to south, and 1050 km from east to west. The total area is thus about 2,176,000 square km, or 50 times that of Denmark. The interior of the country is covered by the inland ice, or ice cap; its thickness in the middle has been cauculated to be 2700 m. The ice-free stretches along the coasts measure about 342,000 square km. Greenland is a typical mountainous country with only a few level areas, to be found especially at the bottom of the fjords. The coast is a characteristic skerry coast with countless large and small islands and deep fjords extending far into the country, often as far as to the inland ice.

Climate.

The whole of the country is north of the 10° isotherm, and thus has a polar climate; owing to the vast extent of the country there are, however, essential differences in temperature and climate. The southern areas of Greenland thus have average temperatures above zero, whereas the northernmost inhabited part of the country has an annual average temperature of about —10° C. (14° Fahr.). Owing to the changing topography of the country, the weather conditions may vary considerably, even within small areas, for instance from mouth to bottom of a fjord. This climate in conjunction with the soil determines a rather scattered and scanty vegetation and, consequently, a fauna of few terrestrial animals. In contrast with this the surrounding sea has a much more abundant fauna of fish, natatorial birds, seals and whales. The sea around Greenland is cold, but there is an essential difference from east to west. A cold stream filled with huge masses of ice passes along the east coast; it is difficult to pass here, so voyage is possible only a few months of the year. The most southern part of West Greenland is touched by a branch from the Gulf Stream. The water is somewhat warmer here, and the harbours are therefore navigable most of the year. Farther north, fjords and bays become ice-bound in winter and do not become navigable again until late in summer. During the last generation, however, the climatic conditions have changed; the temperature of both air and water has risen, and this has been of great importance to coastal navigation and trading.

There are neither roads nor railways in Greenland; consequently all traffic and transport must take place by boat, or in winter in North and East Greenland by sledge.

Population.

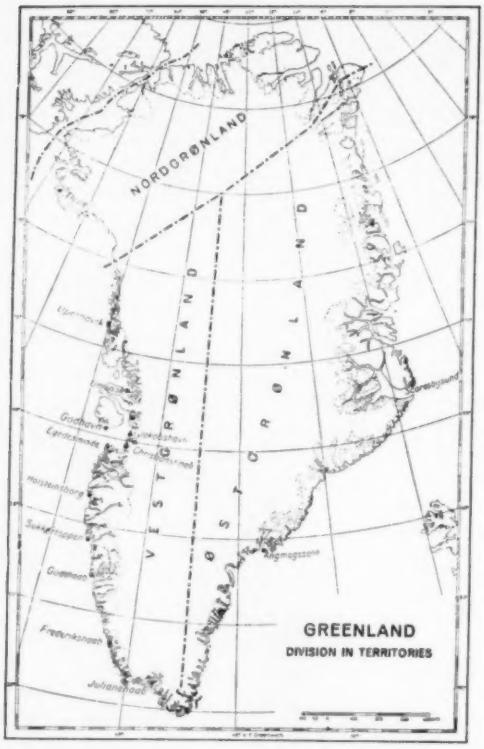
The original population of Greenland were Eskimoes who immigrated centuries ago from the North American continent. In the course of time this population became of more or less mixed blood with a sprinkling of Europeans, especially Scandinavians. This mixed breed now populates especially the middle west coast, whereas a considerable number of Eskimoes still live in the most southern and most northern parts of West Greenland. The sparse population in North Greenland (Thule) and along the east coast is chiefly of Eskimo race. Even though the population of Greenland has increased quite considerably during the last few centuries, they are still few in number as compared to the size of the country, and people live scattered in numerous small settlements along the vast stretch of coast. The density of population is thus only 0.07 inhabitant per square kilometre of ice-free territory. The inhabited places are all in close vicinity to the sea.

Occupations.

The population previously made a living by hunting birds, seals, small whales, and the few terrestrial animals, whereas a considerable change in trade has taken place now from hunting to fishing, as the sea around Greenland has become increasingly abundant in fish owing to the change in climatic conditions. This again has caused a change in nutrition from the more high-protein diet of natural produce to food chiefly composed of carbohydrate. The calorie requirements have largely been covered by this, but the present type of diet is hardly fully sufficient with regard to such necessary components as protein, vitamins and minerals.

Hygiene.

Some towns have sanitary regulations, but in most cases it is difficult to carry these into effect. There is no actual system of removal of refuse and night-soil; only few private houses have lavatories, and very few have drains. There are public lavatories in very few places. The water supply is generally obtained either from wells in the inhabited areas or from neighbouring



small lakes and brooks, where contamination is common. The biological self-purifying property of the earth is very slight owing to the low mean temperature.

Greenland is now divided for administrative purposes into North Greenland (Thule), East Greenland (Angmagssalik and Scoresbysund) and West Greenland. The last territory is subdivided into 16 parishes (see the map).

The country has 14 medical districts, each with a hospital of its own.

PREVIOUS POLIO EPIDEMICS IN GREENLAND

During the last one hundred years poliomyelitis has occurred repeatedly in the form of characteristic epidemics in Greenland. It appears from "Grønlandsk medicinsk statistik og nosografi"*) that the first communication from Greenland on this disease was given in the report of 1858 from Julianehaab medical district.

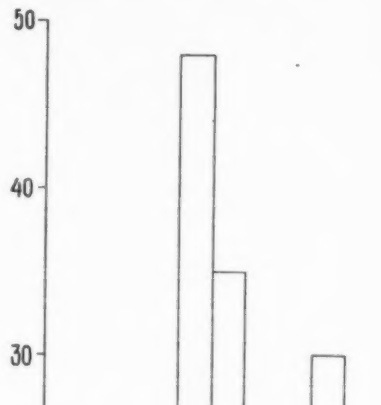
In the present century at least four epidemics are known, whereas actual sporadic cases of poliomyelitis have hardly been observed in Greenland.

The first of these epidemics broke out in September, 1913, at the cryolite quarry of Ivigtut and, during the next summer, spread widely northwards to Godthaab and Sukkertoppen medical districts. It appears from medical reports that the disease was of highly different occurrence in Sukkertoppen and Godthaab, medical districts; in the former there was a widespread epidemic with at least 40 deaths. In the latter the cases were scattered, and only one patient died.

From July to August, 1920, several scattered cases of poliomyelitis occurred in Egedesminde district, and about the same time there were a few cases in Jakobshavn, Christianshaab and Ritenbenk. All cases were mild, and no deaths are known in connection with this epidemic. In great contrast with this, the polio epidemic in Angmagssalik district in 1925 took a very serious course. It has been described by a physician who arrived in the district 2 years later; the description is thus based exclusively on information given by the population at this later date. The epidemic had broken out about the middle of August; almost all Greenlanders were attacked

*) *Alfred Bertelsen:* »Meddelelser om Grønland«, vol. 117, No. 4).

Graphic Representation According to Sex and Age-groups of Total Number of Paretic Cases and Deaths.)*



*) The hatched columns show the number of deaths in the separate age-groups.

and 26 or 27 died of the disease. The symptoms were: A rise of temperature, intense headache, nuchal rigidity and pain in the neck and the back, as a rule constipation, in a few cases diarrhoea, and, further, there was a varying degree of paralysis of the extremities. A few patients made a complete recovery, but persistent paralysis was present in 10. Chiefly young and middle-aged persons were affected, the average age of those who died being 35 years; this was much above the average age in the epidemic in West Greenland in 1913—14. Finally, there was a great epidemic during the period from June, 1932, to November, 1933, in the districts of Sukkertoppen, Holsteinsborg, Godthaab and Egedesminde. The epidemic in Sukkertoppen began on June 16th, 1932, and rapidly spread to the whole of the surrounding district. The last case occurred on August 7th the same year at the outstation Napassok. In less than 2 months 55 clinically manifest cases with 13 deaths occurred among the 538 inhabitants of the district. The cases developed rapidly, one after another, most within a week in any one place. In the adjacent Holsteinsborg district, north of Sukkertoppen, the epidemic broke out soon after with almost the same morbidity and lethality. It thus took a very rapid course and was very widespread. In the separate places the deaths were about twice as frequent among the cases of the first week as among those developing later. Only a very small number of infants had clinically manifest attacks; the average age of 31 patients was 8 years. None of the individuals who lived during the polio epidemic in 1914 were attacked during this epidemic.

In the beginning of January, 1933, the epidemic appeared in Godthaab town. It was thought that the contagion was conveyed by healthy carriers from the settlement of Sukkertoppen. The last communication between the latter and Godthaab was on November 20th; during the intervening period nothing had occurred which might suggest suspicion of poliomyelitis. The epidemic ceased in the town of Godthaab on January 20th, 1933, but four months later the disease appeared in more distant localities, which had not communicated with the town for 3 months after the epidemic ceased there.

Towards the end of 1933 the disease spread to the southern part of Egedesminde and ceased here.

Alfred Bertelsen suggested the possibility that in former times poliomyelitis might have occurred occasionally in Greenland without being recognized at the time of the epidemic, either because it took a very mild course or because there was no physician in the district at the time of the epidemic. As late as in 1945 there was presumably a small epidemic in the Nanortalik area in South Greenland. This epidemic was apparently very mild. Alfred Bertelsen summarized: —

"Epidemics of poliomyelitis have occurred several times in Greenland, whereas it is doubtful whether actual sporadic cases of the disease have ever occurred there.

The outbreak of the epidemics could be naturally connected with foreign navigation, though the contagion must have been conveyed every time with healthy persons as intermediaries. The spread of the disease in Greenland is also effected by healthy carriers, at any rate in the great majority of cases. Direct infection from clinically typical cases is not conspicuous; the disease does not attack members of the same family in particular (at least not in the form of several paretic cases in the same houshold).

The epidemics have been most frequent during the months of late summer, and here the transmission of the contagion by crews of foreign ships may be supposed to be contributory.

The epidemics begin with numerous abortive cases; this is generally followed by an accumulation of severe paralytic cases, after which the clinical picture becomes less severe again. Children under 2 years of age are only seldom severely affected.

The period of incubation may be 24 hours; the question whether it may be longer is difficult to answer owing to the possibility of healthy carriers between the apparent source of infection and a person attacked later on.

The epidemics pass off rapidly in the separate districts; in the small settlements an epidemic generally lasts for about ten days. Most frequently about a year passes from the transmission of the infection to the cessation of the disease in Greenland.

The morbidity is apparently almost universal within the age-groups born after the last epidemic in the locallity concerned; even the abortive type of the disease seems to produce permanent immunity to subsequent exposure.

The lethality in poliomyelitis in Greenland has varied from 0 to 4 per cent of the population in the districts attacked. In paralytic cases it was about 20 per cent. Furthermore, about 25 per cent of such cases resulted in considerable persistent paresis".

THE POLIO EPIDEMIC IN 1952—53

Within a few days about the middle of November, 1952, 3 patients with flaccid pareses were admitted to the hospital in Godthaab. During the days that followed, and gradually decreasing in the course of 3 weeks, there occurred chiefly among children a very considerable number of cases of a disease with the following symptoms: Headache, pain in the neck and the back, often in the extremities, too, spinal rigidity, slight pyrexia for a few days, sometimes nausea, diarrhoea or constipation, and, in some cases, catar-

Locality affected	First case observed	Duration of epidemic in weeks	Population	Number of paretic cases	Deaths
Godthaab	Nov. 15, 1952	5	2834	8	1
Sukkertoppen...	Dec. 8, 1952	6	2058	8	2
Umanak	Jan. 22, 1953	19	1656	21	1
Holsteinsborg...	Feb. 14, 1953	4	1855	2	1
Egedesminde ...	May 14, 1953	12	2870	52	11
Nanortalik	July 13, 1953	1	1068	4	1
Julianehaab	July 14, 1953	3	3723	12	3
Jakobshavn	Sept. 12, 1953	5	1727	17	—
Godhavn	Sept. 25, 1953	7	649	16	1
Qutdligssat	Oct. 2, 1953	1	1343	4	1

rhal affection. The patients remained relatively uninfluenced and generally became spontaneously symptomless in the course of 1 week at most. A general statement of these cases was not possible and, therefore, an arbitrary number of aparalytic cases of poliomyelitis, namely 9, were notified. Pleocystosis was found in 2 of these patients. 8 had paresis, 4 from Godthaab, 3 from the surrounding district, and one who had arrived from Sukkertoppen immediately before the quarantine became effective. One out of these 8 patients developed respiratory paresis, was treated with tracheotomy and overpressure ventilation, but died soon after. The last paretic case occurred on December 19th.

No striking topographical distribution or definite route of infection was found either in the town of Godthaab or in the surroundings. It may, however, be mentioned that 3 men, whose occupation was the emptying of latrine pails ("elsan lavatories"), lived in the houses from which 3 paretic children had been admitted to hospital. There was no pronounced morbidity in the poor district of the town, "Islandsdalen".

Almost at the same time several cases resembling poliomyelitis were notified from various localities in Greenland; for instance from Narssak in South Greenland: A man, aged 29, suddenly became ill (on November 25th, 1952) with headache, slight diarrhoea and a rise of temperature to about 38.°5 C. (101.° F.). Next day his temperature was normal and he was feeling well, but already next morning it rose again. The patient was prostrate, confused, restless, complaining of headache and pain in the abdomen and in the arms. He had painful spasms of the abdominal muscles and brownish vomits. At noon on the same day he had a transient facial palsy; later he had paresis of the right arm and in the course of the day increasingly massive paresis. Towards evening the patient died of respiratory paralysis. He had had nuchal rigidity, though not pronounced, and the temperature did not exceed 38.°5 C. (101.°3 F.). Examination of the spinal fluids was not possible.

At the same time a similar case occurred at Frederikshaab while there was a small epidemic with the following symptoms: A rise of temperature of the "saddle pattern", headache, gastro-enteritis, and diffuse, often rather intense, pain in arms and legs. Some of these patients were admitted to hospital for observation, but the symptom of slight nuchal rigidity mentioned above was only found in 2 cases. Examination of the cerebrospinal fluid in these two patients showed a slight increase in the content of cells (30/3 and 35/3 respectively), but otherwise nothing abnormal.

In the town of Julianehaab, which is near Narssak mentioned above, 2 young children were taken ill, and an infant in the town of Egedesminde in North Greenland was also attacked by the disease. In all three cases the diagnosis was aparalytic poliomyelitis. At that time none of these cases gave rise to an actual epidemic of poliomyelitis.

The table above shows the time of outbreak of the epidemic, its duration in the various localities, the population, the number of paretic cases and of deaths.

It appears from the table that, geographically, a very large area was affected by the epidemic. Only the most northern inhabited places and the whole of the east coast of Greenland were not involved. Morbidity and lethality varied from one district to another. With but one exception the epidemic only attacked the native Greenlandic population.

Towards the end of November and throughout December, 1952, a number of mild cases resembling influenza and characterized by symptoms of gastro-enteritis and pain in the back and the head occurred at Sukkertoppen. In the course of December, 1952, and during the first half of January, 1953, 8 patients in all with paresis were notified; 2 died already on the first day of the disease. There were only 2 patients of the town of Sukkertoppen, the others were resident in the surrounding district, where the two deaths occurred.

In February a number of mild cases, termed epidemic myalgia, occurred in the town of Holsteinsborg. On February 19th the physician was sent for from the outstation Avsakutak, situated an hour's voyage from the town. A boy, aged 15, was said to be ill with a sore throat and had been so for 5 days. He had poliomyelitis of the bulbar type and died the next day. About a fortnight

Paretic Cases and Death) in Polio Epidemic Distributed According to Locality Affected, Sex and Agegroups.*

Locality affected	0–4 years		5–14 years		over 14 years		Total		Total both sexes
	M	F	M	F	M	F	M	F	
Godthaab	—	1	1	2	1	3 (1)	2	6 (1)	8 (1)
Sukkertoppen	—	—	3 (1)	2	1 (1)	3	4 (2)	4	8 (2)
Umanak	2	—	9	2 (1)	5	3	16	5 (1)	21 (1)
Holsteinsborg	—	—	1	—	1 (1)	—	2 (1)	—	2 (1)
Egedesminde	3	1	19 (4)	18 (4)	1	10 (3)	23 (4)	29 (7)	52 (11)
Nanortalik	1	—	2 (1)	1	—	—	3 (1)	1	4 (1)
Julianehaab	1	—	2 (1)	4	3	2 (2)	6 (1)	6 (2)	12 (3)
Godhavn, Jakobshavn and Qutdligssat	4	—	11 (1)	6	6 (1)	10	21 (2)	16	37 (2)
Total	11	2	48 (8)	35 (5)	18 (3)	30 (6)	77 (11)	67 (11)	144 (22)

*) Number of deaths given in parenthesis.

later a boy, aged 6, with aparatytic poliomyelitis was seen at the oustation Sarfanguak. Another 14 days later a paralytic case of the disease was seen at the settlement Sarkardlit. Two brothers of the last patient had also been ill; one with symptoms of the ear had died a fortnight earlier; it had not been possible to send for the doctor then. The second brother had presumably had an aparalytic poliomyelitis. Finally, according to information given by the inhabitants, there had been 5 cases of presumably aparalytic poliomyelitis in children at ages from 3 to 12 years at a fourth settlement. The epidemic in the medical district of Holsteinsborg thus passed off solely in the surrounding district, and its course must be termed very mild.

In the beginning of December two motor-boats arrived at the town of Holsteinsborg; they represented the last communication with the district of Sukkertoppen farther south. They were quarantined in the harbour, as the first case of poliomyelitis at Sukkertoppen had just been reported.

The first case of the disease thus occurred over 2 months after Holsteinsborg had last been in contact with other localities; it was the case of a young man, who had not left the territory of the settlement since his return, 6 months earlier, from North Greenland. All the other patients had also remained in the settlement for a long time.

At the end of January the epidemic appeared in the district of Umanak far north in Greenland; on January 22nd, 3 cases were ascertained in the town of Umanak — all boys with paresis of the lower extremities. At this time 40 days had passed since the schooner of the town had arrived from one of the southern districts. The passengers on board the ship included a family who had visited Sukkertoppen in November. It may be mentioned that the first patient attacked by poliomyelitis was a brother of the head of the family returning by the schooner, and that all 3 cases, as well as 2 occurring later on, were living near his house. Within barely 3 weeks there occurred 24 cases of poliomyelitis, including 5 with paresis, in the town of Umanak.

The day before the first case was ascertained, two men from a neighbouring settlement had visited Umanak. There had been no other communication between these localities since the end of November. 48 days later the first case of poliomyelitis occurred in this settlement. Eight cases in all, 4 with paresis, occurred here. In spite of repeated warnings there had been a rather brisk traffic by sledge between various outstations and settlements in the surrounding district, and during the months that followed the epidemic spread here. It died away about June 1st.

The polio epidemic reached its climax in the area at Disko Bay, where all four medical districts, Egedesminde, Jakobshavn, Godthaab and Qutdligssat were involved in the course of about 6 months.

The first cases appeared simultaneously in the town of Egedesminde and at Kangatsiak. During the first week of the epidemic 9 patients were admitted to hospital; already within the first three days of the epidemic 4 of these patients died with symptoms of bulbar or respiratory paresis.

In the second week the morbidity decreased considerably, but then increased and reached its climax in the seventh week; this was followed by an abrupt fall until the epidemic ceased in the twelfth week.

At one outstation with a population of 128 individuals, living in small and crowded houses, the course of the epidemic was explosive. In the course of 4 days 14 patients were admitted to hospital from this place; 5 were aparalytic, 9 were paretic cases, and 2 of the latter died.

Nine of these patients, 8 with paresis, were under 15 years.

Of the 17 inhabited places in the district, 14 were involved in the epidemic. In the town of Egedesminde the cases appeared with almost the same frequency throughout the duration of the epidemic, over 2 months; in the surrounding country the epidemic passed off more rapidly and seldom lasted more than from 8 to 14 days.

During the period from May 14th to August 3rd 75 polio patients in all were admitted to the hospital at Egedesminde: 67 had pleocytosis in the cerebrospinal fluid, and the other seven were

admitted with paresis ascertained at a follow-up in the surrounding country. 52 had paresis, and 11 of these died with symptoms of respiratory or bulbar paresis. The great majority of those admitted, 23 boys and 23 girls, were of the age-group 5—15 years. 3 were over 25 years, 4 of the women, all with paresis, were pregnant. One, in the fourth month of pregnancy, died in 3 days; the three others, in the third, fifth and seventh months respectively, later had normal deliveries.

On the whole, the further course of the epidemic in the Disko Bay was of a less serious nature, though several patients were severely attacked, but there were only a few deaths.

In the beginning of September the first case of poliomyelitis occurred in Jakobshavn. There were a number of cases suspected of mild attacks of the disease, and a total of 17 paretic cases, but no deaths. The great majority of cases occurred in the town of Jakobshavn and in settlements in the immediate neighbourhood, whereas there was only one case in the neighbouring settlement of Rodebay in the whole territory north of Jakobshavn.

In Godhavn the epidemic lasted for 7 weeks from the end of September. Sixteen paretic cases were notified; in contrast with those occurring in the districts of Jakobshavn and Qutdligssat, these cases were scattered throughout the whole area of Godhavn.

In the medical district of Qutdligssat, cases of poliomyelitis occurred only in the coal-mining town proper of the same name; 7 became ill, all in the course of one week. Four of these patients were severely paretic. A boy, aged 16, died of respiratory paresis in the course of conveyance from Qutdligssat to Jakobshavn. The three other paretic patients from Qutdligssat and a great number of the patients from the Godhavn district were taken to the hospital at Jakobshavn, where the treatment of the most serious cases from these areas had been concentrated.

It proved impossible to demonstrate a definite source of infection in the case of the epidemic in the areas at Disko Bay. At Egedesminde it broke out soon after the arrival of the first ship from Denmark, but no information is available on persons infected with poliomyelitis on board this ship. During the months of spring and summer, however, there is much intense intercourse between the various districts of Disko Bay and, even though it was attempted by quarantine regulations to prevent all communication, there has undoubtedly been uncontrollable sailing in small vessels between the minor settlements.

In the middle of July the epidemic appeared in South Greenland. In the town of Nanortalik a boy, aged 10 years, suddenly became ill, and died 2 days later of respiratory paresis. The next day the second case occurred in the town of Narssak in the Julianehaab district. This patient was also a boy, aged 11; the course of the disease was fulminant and proved fatal in 4 days. In the course of the week that followed there were further 3 paralytic and a few aparalytic cases in the district of Nanortalik. The epidemic then ceased in this area.

The district of Julianehaab had a total of 12 paralytic cases and 3 deaths. The cases appeared quite irregularly both in the district of Julianehaab and in that of Nanortalik, apparently without epidemilogical continuity. A definite source of infection was not found here either. Three cases of herpes were observed during the epidemic in Nanortalik.

In the endeavours to counteract the epidemic in the various districts, quarantine regulations were introduced with a quarantine period of 14 days, assembling was prohibited, and school and church attendance, cinema performances, meetings, etc., were called off.

The population were requested over the wireless and through the papers to carry through strict cleanliness and to avoid excessive exertion and over-indulgence in alcohol. Two polio teams of specially trained physicians, nurses and physiotherapists were sent up from Denmark to assist in counteracting the epidemic. Emergency hospitals were set up in different parts of the country in order thus to concentrate the treatment of the patients most severely attacked, and is was attempted to establish a regular ambulance service by putting in boats to take patients to hospital as quickly as possible. Special equipment in the form of respirators, surgical equipment, cylinders with compressed air and oxygen, etc., was placed at disposal. Several patients with respiratory paresis were treated with tracheotomy and overpressure ventilation, and later continued with respirator treatment.

After-treatment of the polio patients in Greenland was undertaken either in Copenhagen or in Greenland. The severest cases, 64 patients in all, were taken to Denmark by hospital ship for additional treatment in special departments here, whereas the milder cases could be dealt with in the local hospitals in Greenland under the management of specially trained physiotherapists sent up there.

EPIDEMIOLOGICAL INVESTIGATIONS

A great number of faecal samples and blood samples were sent from the greater part of Greenland to Statens Seruminstitut, Copenhagen, for virus determination and antibody determination, respectively.

However, most of the blood samples proved to be toxic to tissue culture, presumably owing to difficulties in storing and forwarding; therefore they could not be examined for antibodies. But polio virus of Type I was demonstrated in several patients from Julianehaab, Egedesminde, Jakobshavn, Qutdligssat and Godhavn.

SUBJECT INDEX

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ERRATUM: Line 10—15 (incl.) from below in the left-hand column of page 184 belong in the left-hand column of page 182, between line 21 and 22 from above.

NOTE: The bibliographial Supplement INDEX MEDICUS DANICUS should not be bound with the DANISH MEDICAL BULLETIN, as it is paginatep separately and will be continued independently.